Molecular imaging as a tool to investigate heterogeneity of advanced HER2-positive breast cancer and to predict patient outcome under trastuzumab emtansine (T-DM1): the ZEPHIR trial

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Molecular imaging as a tool to investigate heterogeneity of advanced HER2-positive breast cancer and to predict patient outcome under trastuzumab emtansine (T-DM1): the ZEPHIR Trial

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Key Message: ""Molecular imaging of metastatic HER2 positive breast cancer reveals marked intra- and inter- patient heterogeneity in HER2 mapping which correlates with clinical outcome under TDM1 therapy""

Abstract

Background

Only human epidermal growth factor receptor (HER)2-status determined by immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) has been validated to predict efficacy of HER2-targeting antibody-drug-conjugate trastuzumab-emtansine (T-DM1). We propose molecular imaging to explore intra-/interpatient heterogeneity in HER2-mapping of metastatic disease and to identify patients unlikely to benefit from T-DM1.

Patients and methods

HER2-positive mBC patients with IHC3+ or FISH ≥ 2.2 scheduled for T-DM1 underwent a pre-treatment HER2-PET/CT with ^{89}Zr-trastuzumab. FDG-PET/CT was performed at baseline and before T-DM1 cycle 2. Patients were grouped into four HER2-PET/CT patterns according to the proportion of FDG-avid tumor load showing relevant ^{89}Zr-trastuzumab uptake (> bloodpool activity): patterns A and B were considered positive (>50% or all of the tumor load "positive"); patterns C and D were considered negative (>50% or all of the tumor load "negative"). Early FDG-PET/CT was defined as non-responding when > 50% of the tumor load showed no significant reduction of FDG-uptake (< 15%). Negative and positive predictive values (NPV, PPV) of HER2-PET/CT, early FDG-response and their combination were assessed to predict morphological response (RECIST 1.1) after three T-DM1 cycles and time to treatment failure (TTF).

Results

In the 56 patients analyzed, 29% had negative HER2-PET/CT while intrapatient heterogeneity (patterns B and C) was found in 46% of patients. Compared to RECIST1.1, respective NPV/PPV for HER2-PET/CT were 88%/72% and 83%/96%

for early FDG-PET/CT. Combining HER2-PET/CT and FDG-PET/CT accurately predicted morphological response (PPV and NPV:100%) and discriminated patients with a median TTF of only 2.8 months (*n*=12, 95% CI: 1.4-7.6) from those with a TTF of 15 months (*n* = 25, 95% CI: 9.7-not calculable)

Conclusions

Pre-treatment imaging of HER2-targeting, combined with early metabolic response assessment holds great promise for improving the understanding of tumor heterogeneity in mBC and for selecting patients who will/ will not benefit from T-DM1.

ClinicalTrials.gov identifier: NCT01565200

Key words: HER2 positive Breast Cancer, HER2 imaging, Prediction of T-DM1 efficacy

Introduction

Trastuzumab-emtansine (T-DM1), a novel human epidermal growth factor receptor (HER) 2 targeting antibody-drug conjugate (ADC), was recently approved for the treatment of advanced HER2-positive breast cancer (BC) patients who progressed after a prior line of trastuzumab-based therapy [1]. T-DM1 combines the HER2-targeting properties of trastuzumab with intracellular delivery of DM1, a potent derivative of the antimicrotubule agent maytansine [2]. To exert antitumor activity, T-DM1 relies on the efficient targeting of HER2 [3], which can sometimes be compromised [4, 5]. Besides HER2 “positivity” no validated predictive markers of response to T-DM1 are available. Upon disease relapse, current guidelines recommend a biopsy to check the current molecular profile of the tumor [6, 7]. However, this invasive biopsy may not always be contributive or be a reliable reflection of the receptor status of the entire tumor load [8].

Molecular imaging might serve this purpose based on previous clinical experiences: (1) the successful use of early FDG-PET/CT for predicting the likelihood of pathological complete response was demonstrated in early HER2-positive BC [9]; (2) early FDG-PET/CT response criteria based on the assessment of metastatic tumor load were developed and correlated with patient outcomes in advanced colorectal cancer [10] and (3) administration of zirconium-89 (^{89}Zr) radiolabeled trastuzumab allowed the detection of HER2-positive lesions on PET/CT in patients with HER2-positive metastatic BC (mBC) [11].

We therefore propose the use of molecular imaging to improve T-DM1 individualization in patients with mBC. HER2-PET/CT imaging may provide high predictive values (PVs) for T-DM1 efficacy, however as intrinsic resistance mechanisms to trastuzumab and/or DM1 may well exist [5], the addition of an “early”

metabolic response assessment by FDG-PET/CT after one T-DM1 cycle could provide early identification of non-responding patients or lesions. This might avoid the toxicity and costs of T-DM1 and improve patient outcome by switching sooner to a more optimal therapy [12].

We report a patient-based analysis in which the negative and positive PVs (NPV and PPV) of HER2-PET/CT, early FDG-response before cycle 2 of T-DM1 and their combination were compared to RECIST 1.1 anatomical response evaluation after 3 T-DM1 cycles and correlated with time to treatment failure (TTF).

Methods

Study design, patient's eligibility and treatment

The ZEPHIR study is a multicenter trial enrolling patients from Belgian and The Netherlands eligible to receive T-DM1 for HER2-positive advanced disease. Nation-specific definitions were used for "HER2 positivity": immunohistochemistry (IHC) 3+ or FISH>2.2 in the Netherlands and FISH>2.2 in Belgium. HER2 status was determined in the primary tumor or when not available in a metastasis. Patient's eligibility is described in supplementary material and methods section. In brief, eligible patients had progressive advanced HER2-positive BC pre-treated or not with one or more treatment lines. Imaging of at least two "target" lesions was required with the following criteria: (1) anatomically transaxial diameter ≥ 1.5 cm and measurable per RECIST1.1. and (2) metabolically assessable[13] with a maximum standard uptake value (SUVmax) corrected for lean body mass ≥ 1.5 x SUVmean + 2 standard deviations (SD) of the liver measured in a 3 cm diameter spherical volume of interest (VOI) in normal liver parenchyma.

Following pre-treatment imaging with ^{89}Zr-trastuzumab-PET/CT (HER2-PET/CT), patients received 3 cycles of T-DM1. At baseline and after 3 cycles of T-DM1, tumor response was assessed anatomically according to RECIST1.1 with CT [14]. An "early" metabolic response assessment with FDG-PET/CT was performed just before cycle 2. The HER2-PET/CT and "early" FDG-PET/CT results were blinded to the treating oncologist (Supplementary figure 1).

T-DM1 dosed 3.6 mg/kg was administered intravenously every 3 weeks (21 days +/- 3 days). Patients were followed until treatment discontinuation resulting of disease progression, unacceptable toxicity or patient withdrawal from the study. In case of toxicity the dose could be reduced to 3 or 2.4 mg/kg. Toxicity was scored according to the National Cancer Institute Common Toxicity Criteria V4.0.

The ethics committee and relevant health authorities at each participating institution approved the study protocol (ClinicalTrials.gov identifier: NCT01565200). All patients gave written informed consent.

Imaging procedures

FDG-PET/CT procedures described in supplementary appendix were based on European Association of Nuclear Medicine (EANM) recommendations[15].

For HER2-PET/CT, patients were injected with 37 MBq (+/- 10%) ^{89}Zr-trastuzumab and 50 mg cold trastuzumab 4 days before acquisition [11]. Tracer preparation and standardization of image acquisition/reconstruction were performed as reported earlier [16].

The protocol allowed maximum 21 days between baseline imaging and treatment start. Early FDG-PET/CT had to be scheduled in the week preceding cycle 2.

An imaging core lab (Orilab, Institut Jules Bordet, Brussels) assessed the image quality and compliance to the imaging guidelines.

Image analysis

Two independent nuclear medicine physicians reviewed the PET images and discordances were revised by a third. Diagnostic CTs were centrally reviewed by a senior radiologist. A visual "patient-based" classification capturing the whole disease burden was developed by using a side by side display, comparing baseline FDG-PET/CT (showing all FDG-positive metastases independent of their HER2-imaging status) and HER2-PET/CT. Patients were grouped into four HER2-PET/CT patterns (A to D) according to the proportion of FDG avid tumor load showing relevant ^{89}Zr-trastuzumab uptake as shown and detailed in figure 1. Patterns A and B were considered "HER2-positive" and patterns C and D "HER2-negative".

T-DM1 response assessment was determined anatomically according to RECIST 1.1. Patients with an objective response (OR) (complete and partial anatomic response) were classified as responders. Those showing stable or progressive disease were classified as non-responders. The early metabolic assessment was based on EORTC criteria [17], with lesion response cut-off set at 15% SUVmax decrease, and progression defined as a 25% increase in SUVmax. Based on previous research [18], a low cut-off value for response for early FDG-PET was chosen to obtain the highest NPV. Patient-based response classification is described in detail in supplementary figure 2. In brief, patients were grouped into class I (all lesions show a significant response); class II (mixed response, dominant response); class III (mixed response, dominant non-response); and class IV (no lesion is responding, or, presence of at least one progressive or new lesion) [10]. Classes I

and II were considered as metabolic responses, and classes III and IV as metabolic non-responses.

Statistical Plan

The ZEPHIR trial, being the first prospective and comprehensive imaging study in advanced HER2 positive BC should be considered a “phase I” imaging study, for which a patient-oriented statistical hypothesis was impossible to formulate. The sample size therefore had to be based on a “lesion-based” analysis. The NPV of HER2-PET/CT was chosen as primary objective, requiring 60 RECIST evaluable lesions negative on HER2-PET/CT . This report focuses on the initial sample of 60 patients with the exploratory objective of predicting the patient-based anatomical response after 3 T-DM1 cycles and the TTF using pretreatment HER2-PET/CT combined or not with early FDG-PET/CT response.

TTF is the time from start of T-DM1 to discontinuation for any reason, including disease progression (clinical or image-based), treatment toxicity or death. For the correlation between TTF and imaging results, patients who discontinued T-DM1 for other reasons than progression were censored. Distribution of TTF was estimated by the Kaplan-Meier method. For comparisons, the data were fitted with Cox regression models. Hazard ratios (HRs) are reported with 95% confidence intervals (CI). Statistical significance level was set at 5%.

The planned extension of the Zephir study to reach 101 patients serves two purposes: obtaining the 60 HER2-PET negative lesions, and gaining insight into molecular portraits of “negative” versus “positive” lesions, supported by mandatory biopsies. Of note, a validation of the patient-based analysis presented here would require 300 patients and is beyond the scope of the Zephir trial.

Results

Patients

Sixty patients with a median of 3 prior metastatic treatment lines were included between May 2012 and October 2013, of whom 56 were anatomically evaluable. Four patients were excluded: consent withdrawn (*n*=1), death before starting treatment due to disease progression (*n*=1), absence of target lesions (*n*=1), inferior image quality (*n*=1). Patient characteristics are detailed in supplementary table 1.

Treatment results of T-DM1

Fifty-seven patients received T-DM1.The median number of T-DM1 cycles as of June 2015 was 14.5 (range 2-45), with a median TTF of 7.7 months (95% CI: 5.8-9.7 months) and with eight patients still on trial at the time of analysis. Reasons for discontinuation of treatment were disease progression or toxicity in 88% (43/49) and 12% (6/49), respectively. Dose reduction for toxicity occurred in 12 patients: for thrombocytopenia (*n*=7), neurotoxicity (*n*=2) and increased gamma-glutamyltransferase, neutropenia and general malaise (each *n*=1). Twenty-four serious adverse events were reported in 16 patients, with 11 possibly related to T-DM1, including thrombocytopenia (*n*=2), fever and chills (*n*=4) and perianal abscess, pulmonary hemorrhage, cognitive disturbance, seizure and supraventricular tachycardia (all *n*=1). Objective response rate (ORR) of the 56 evaluable patients, using RECIST1.1, was 55% (31 out of 56) after 3 cycles of T-DM1.

Molecular Imaging results

HER2-PET/CT was performed in 57 patients of whom one was excluded from analysis due to motion artefact. One patient experienced a grade 1 allergic reaction

after ^{89}Zr-trastuzumab administration. Twenty-one patients had an HER2-PET/CT pattern A, 19 pattern B, 7 pattern C, and 9 pattern D, indicating substantial heterogeneity of ^{89}Zr-trastuzumab uptake within these “HER2-positive” patients. After dichotomization, 16 (29%) patients were considered as HER2-PET/CT negative. Furthermore, HER2-PET/CT revealed organ-based heterogeneity of tumor uptake, with the highest uptake in liver metastases (Supplementary figure 3). Significant myocardial uptake was never observed. The dichotomized classification of the HER2-PET/CT was reproducible between observers with a kappa of 0.60.

Anatomical response was assessable in 55 of these patients. HER2-PET/CT was classified positive in 39 patients, of which 28 showed OR after 3 T-DM1 cycles (PPV: 72%). Sixteen patients were classified HER2-negative, 14 of which showed stable or progressive on CT (NPV: 88%) (Supplementary table 2).

Early FDG-PET/CT metabolic response assessment was available in 56 patients: 27 responders (19 class I and 8 class II) and 29 non-responders (11 class III and 18 class IV) were found. Twenty-six of the 27 responding patients showed OR after 3 cycles of T-DM1 (PPV: 96%). Twenty-four of the 29 non-responding patients showed absence of OR (NPV: 83%) (Appendix table 2). Additional exploration of this NPV revealed a median time of 14.5 days (range: 4-31 days) between baseline FDG-PET/CT and first T-DM1 administration. Within this time frame, rapid disease progression before start of T-DM1 could retrospectively be identified: in patient A, metabolic progression was documented between two successive baseline FDG-PET/CT scans performed 16 days apart; while in patient B, laboratory evidence indicated rapid progression between baseline blood analysis (increase in liver enzymes and serum tumor marker) and start of T-DM1 showing a progressive early

FDG-PET/CT, but a clear radiological and metabolic response after 3 cycles of T-DM1 (supplementary figure 3).

When combining both molecular imaging results, all patients showing uptake on the HER2-PET/CT and an early metabolic response showed an OR using RECIST1.1 (PPV 100%). In contrast, patients without substantial uptake on the HER2-PET/CT and showing stable or progressive metabolic early response were all classified as non-responders. Eighteen patients had discordant results (e.g. positive HER2-PET/CT and early non response or vice-versa): the HER2-PET/CT accurately predicted response in only 28% (5/18) of the patients while early FDG-PET/CT was correct in 72% of the cases (13/18).

The molecular imaging results were correlated with treatment discontinuation (figure 2). Median TTF was 11.2 months (95% CI: 8-15 months) in the HER2-imaging-positive group and 3.5 months in the HER2-imaging-negative group (95% CI: 1.4-7.6 months). The HR was 4.5 (95% CI: 2.1-9.4, p<0.0001) using the HER2-imaging-positive group as reference. TTF for patients responding on the early FDG-PET/CT was 13.3 months (95% CI: 9.3 months – not calculable) compared to 4.2 months (95% CI: 1.4-7.6 months) in the non-responding group with HR 3.8 (95% CI: 2-7.4, p < 0.0001) for the non-responding group.

Of interest, combining HER2-PET/CT and early FDG-PET/CT results discriminated a group with a median TTF of 15 months (95% CI: 9.7 months – not calculable), with a positive HER2-PET/CT associated with a response on early FDG-PET/CT, from a group with a worse outcome (median TTF 2.8 months, 95% CI: 1.4-7.6 months), when a negative HER2-PET/CT preceded a non-response on early FDG-PET. Discordant results were linked to an intermediate group with median TTF of 6.3 months (95% CI: 1.4-9.0 months). Using the double positive imaging group as a

reference, the HR for the double negative and the discordant groups were 8.3 and 3.7 (p <0.0001 overall).

Discussion

Our study shows that pre-treatment imaging of HER2 with HER2-PET/CT is a promising tool for studying inter-lesion heterogeneity in advanced disease stages. When combined with early FDG-PET/CT after 1 cycle of T-DM1 it is powerful in predicting which patients will or will not benefit from T-DM1.

The use of two imaging modalities separated patients treated with T-DM1 with a 2.8 versus 15 months median TTF. About one-third of the patients with HER2-positive advanced BC showed little or no ^{89}Zr-trastuzumab uptake across their metastases and experienced a shorter median TTF compared to patients with a more homogenously positive HER2-PET/CT.

TTF was used as an outcome as it closely reflects actual clinical practice, by representing the time point when patient and physician do not see any benefit in continuing the therapy. Progression-free survival (PFS) as an endpoint would have required a strict uniform auditable response assessment beyond 3 T-DM1 cycles, which was beyond the scope of ZEPHIR. Notably, in our study only 6 patients discontinued treatment for toxicity. This is in line with the reported favorable T-DM1 toxicity profile.[19]

The NPV of the HER2-PET/CT in terms of RECIST1.1 response after 3 cycles of T-DM1 was clinically relevant, 88%. Further inquiry is still needed to understand why two patients, classified as negative by the HER2-PET/CT before start of treatment, responded to T-DM1 according to RECIST1.1. Our study did not distinguish between a lack of receptor overexpression, receptor masking [4] or an induced response

despite HER2 low availability due to the extreme potency of DM1 in the absence of intracellular resistance mechanisms in these patients.

The results of the early FDG-PET/CT alone were valuable in predicting clinical outcome: the NPV was 83% in terms of OR after 3 cycles of T-DM1 with a median TTF of 4.2 months, contrasting sharply with the TTF of FDG-PET/CT early-responding patients of 13.3 months. However, even within the short interval (14.5 days) between the baseline FDG-PET/CT and start of treatment a rapid disease progression could be demonstrated which may have led to false negative early metabolic response. FDG-PET/CT performance might be further improved by scheduling the baseline FDG-PET/CT shortly (<7 days) before start of T-DM1.

Our FDG-PET response criteria, based on the whole metastatic tumor load, focus mainly on the identification of treatment-resistant lesions [10]. Clearly, combining HER2-PET/CT and FDG-PET/CT achieved the most powerful discrimination in terms of clinical outcomes: the NPV and the PPV for an OR by RECIST1.1 were both 100% and the corresponding median TTFs differed considerably. In discordant patients (e.g. patients with a “positive” HER2-PET/CT but a non-responding early FDG-PET/CT or vice versa) FDG-PET/CT was more accurate in predicting RECIST1.1 response. A plausible explanation for the contribution of FDG-PET to HER2-PET/CT is that the presence of the target is a prerequisite for the activity of T-DM1 but resistance to T-DM1 can still occur, a phenomenon that will be captured by the early FDG-PET/CT.

The concept of interrupting T-DM1 after one cycle in case of early FDG-PET/CT non-response deserves to be tested in a future randomized trial with cost-effectiveness as secondary endpoint; the cost of incorporating FDG-PET/CT in the treatment plan

would be offset by early interruption of T-DM1 in roughly 50% of the patient population.

Our trial is the first to prospectively evaluate PET imaging as a tool for improved individualization of anti-HER2 therapy in advanced HER2-positive BC. Hence, it can provide important information for the development of new ADCs.

Our trial also shows that molecular imaging deserves more attention in the era of personalized medicine and should be incorporated in translational research efforts in parallel to high-throughput sequencing technologies which provide granular information on a small piece of tumor tissue. The advantages of molecular imaging include non-invasiveness, broad applicability and the ability to evaluate the entire disease burden. Thus the sampling error due to tumor heterogeneity, increasingly acknowledged as challenge for successful therapy, could be avoided.

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Disclosure

GG (first author) has declared conflict related to an immediate family member (Leadership with Pharamar company; Honoraria with Roche/Genentech company; Advisory Role with Roche/Genentech company) and research funding from Roche/Genentech.

SS (author 7) has received research funding from Johnson&Johnson company (not related to the conduct of this study)

MH (author 8) has declared consulting or advisory role and honoraria from Esai, Roche/Genentech and AstraZeneca company as well as Speakers' Bureau with Roche/Genentech company (not related to the conduct of this study).

JT (author 10) has declared travel/accommodations grants with Novartis and Pfizer (not related to the conduct of this study).

WO (author 11) has declared honoraria, consulting fees, research funding and speakers' Bureau with Bayer (not related to the conduct of this study).

C.W.M (author 15) has declared consulting or advisory role with Amgen and Esai as well as travel/accommodations fees with Amgen and Merck.

TG (author 16) has declared research funding with Servier, Elro Pharma, arGEN-X BVBA and Synta as well as travel/accommodations/expenses with General electric.

AA (author 18) has declared consulting or advisory role with Roche/Genentech, Nektar and Bayer.

E.d.V (author 19) has declared research grants from Roche/Genentech, Amgen, Novartis, Pieris and Servier to the institute, data monitoring committee Biomarin, advisory board Synthon.

PF (author 20) has declared consulting /advisory role and Speakers'Bureau with Bayer and Sirtex as well as research funding from Roche/Genentech, Sirtex and Bayer.

Figure 1: Patterns of HER2-PET/CT confronted with FDG-PET/CT, Maximum intensity projection.

Lesion uptake was considered pertinent when visually higher than bloodpool. Pattern A: entire tumor load showed pertinent tracer uptake; B: dominant part of tumor load showed tracer uptake; C: minor part of tumor load showed tracer uptake; D: entire tumor load lacked tracer uptake.

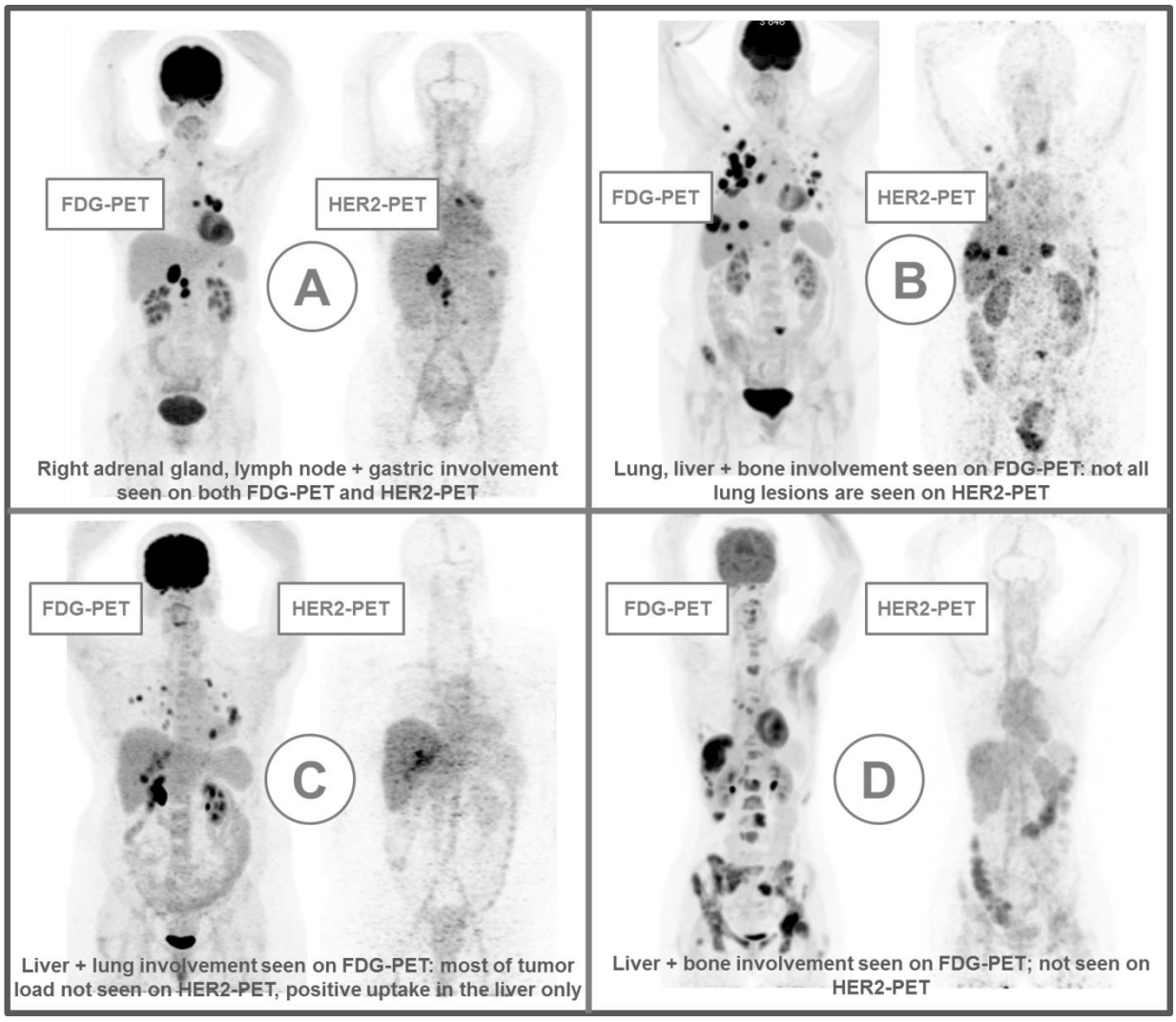
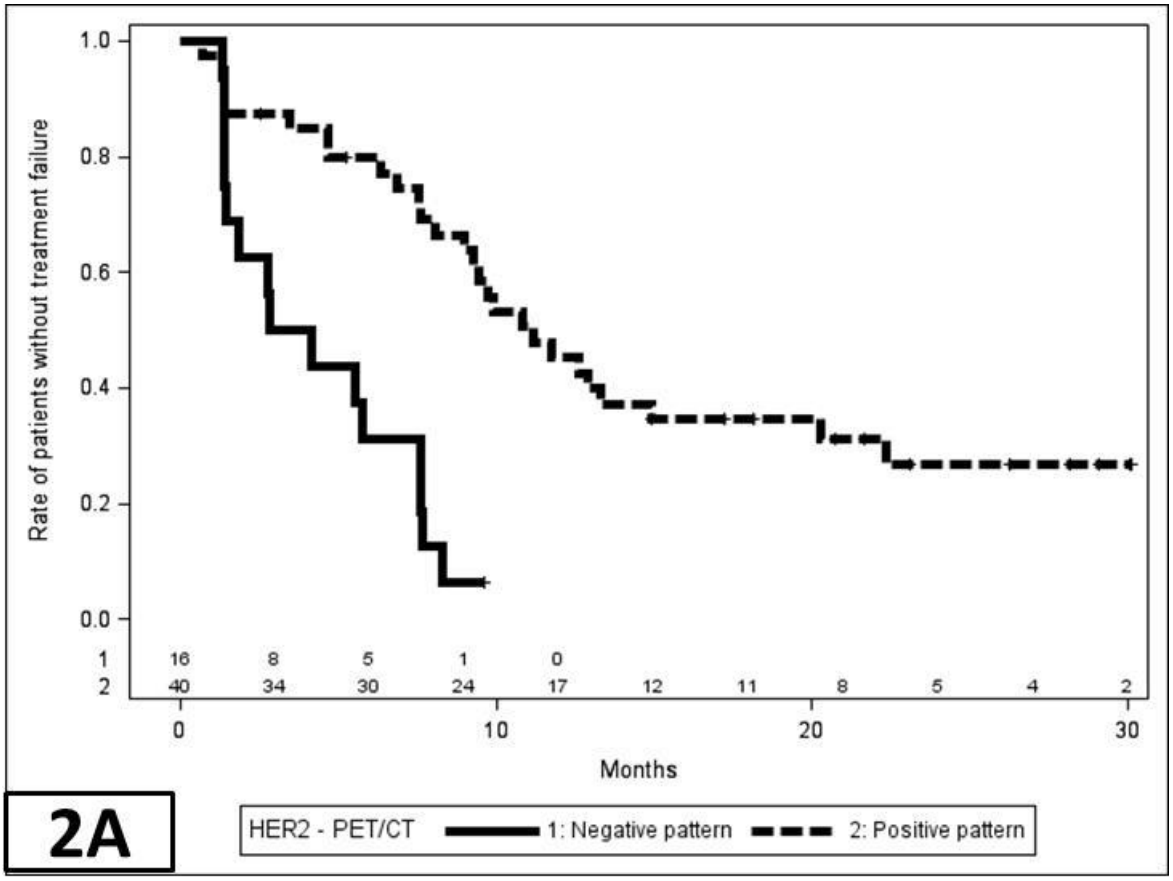


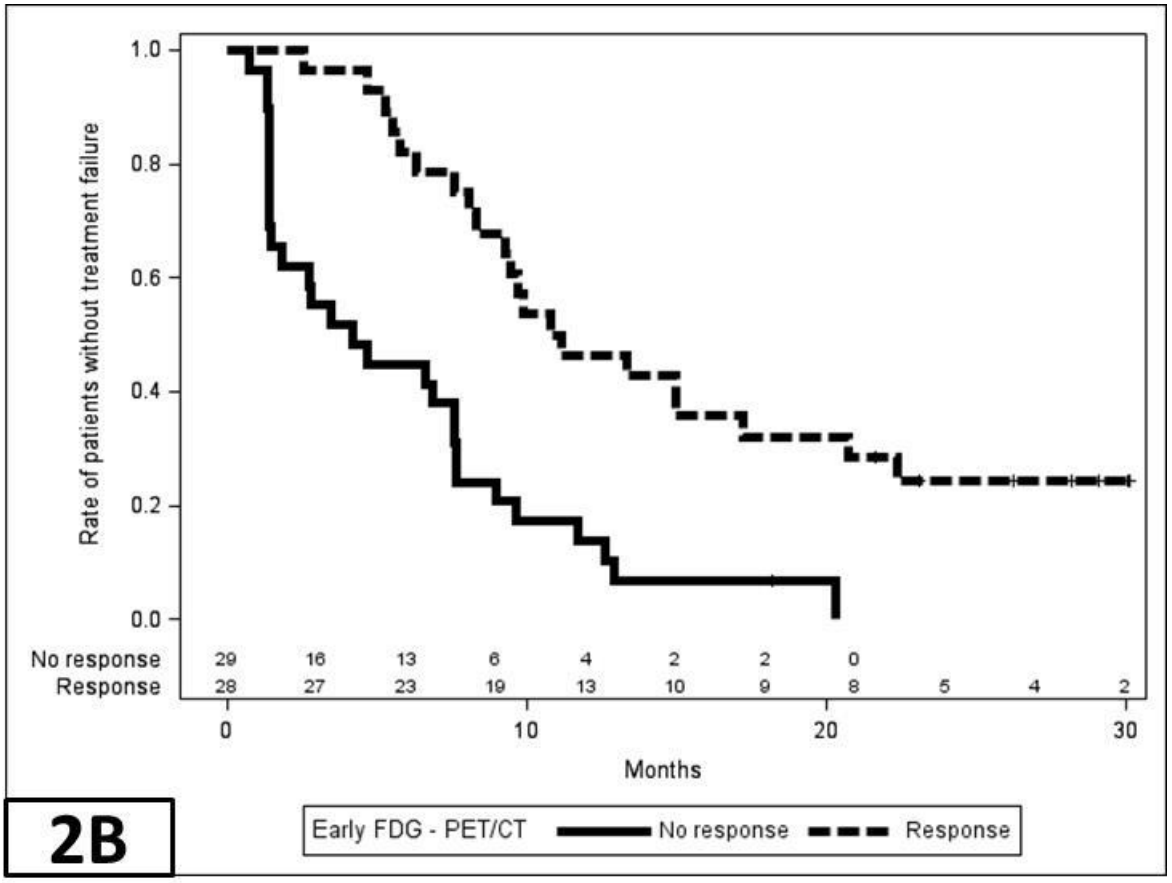
Figure 2: Time to treatment failure according to HER2-PET/CT alone (2A); early FDG-PET/CT alone (2B); combination of the HER2-PET/CT and early FDG-PET/CT (2C).

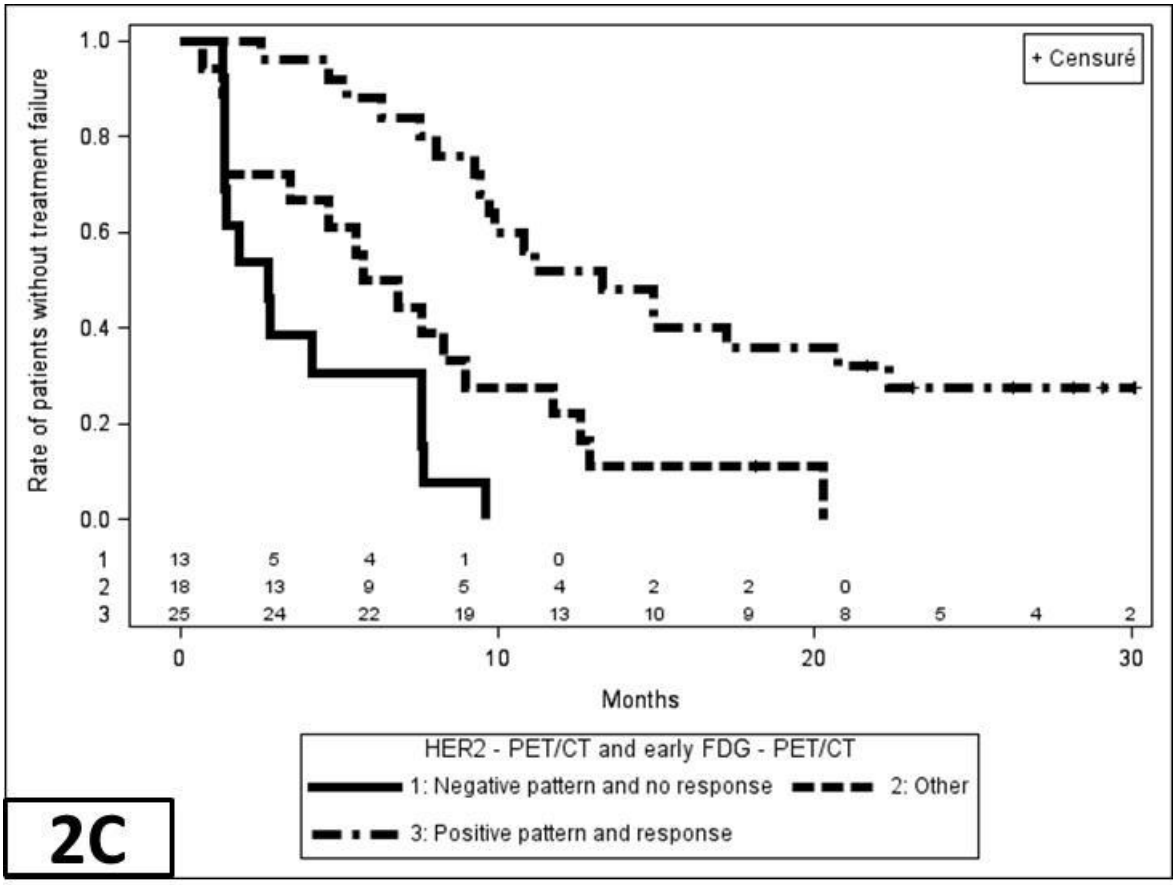
Figure 2A: Patients with pattern A and B for HER2 uptake (positive pattern, dashed line). Patients with pattern C and D HER2 uptake (negative pattern, full line).

Figure 2B: Patients with early metabolic response on FDG-PET/CT (Response, dashed line). Patients without early metabolic response on FDG-PET/CT (No response, full line)

Figure 2C: Patients with positive pattern on HER2-PET/CT showing early metabolic response on FDG-PET/CT (dashed-dotted line); Patients with negative patterns on HER2-PET/CT without early metabolic response on FDG-PET/CT (full line); Patients with positive patterns on HER2-PET/CT and early non response or vice-versa (discordant cases) (dashed line)







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